

REPLY

Serial No. 10/712,525  
Atty. Docket No. GP123-03.DV1

Amendments to the Specification

**The specification is amended at page 4, lines 14-25, as follows:**

In addition to anionic groups, polynucleotide probes featured in the present invention may further include cationic and/or nonionic groups, provided the probes have a net positive negative charge. The polynucleotide may consist of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), a combination of DNA and RNA, or it may include a nucleic acid analog (e.g., a peptide nucleic acid) or contain one or more modified nucleosides (e.g., a ribonucleoside having a 2'-O-methyl substitution to the ribofuranosyl moiety). Non-nucleotide groups, such as polysaccharides or polyethylene glycol, may also be included in the probes, provided they do not prevent or substantially interfere with hybridization of the probe to the target nucleic acid. Probes of the present invention are up to 100 bases or more in length (preferably from 12 to 50 bases, and more preferably from 18 to 35 bases in length) and contain a base region which is complementary to a target sequence contained in the target nucleic acid (the base region is preferably perfectly complementary to the target sequence).

**The specification is amended in the paragraph bridging pages 23 and 24 as follows:**

A preferred method for determining  $T_m$  measures hybridization using the Hybridization Protection Assay (HPA) disclosed by Arnold *et al.*, "Homogenous Protection Assay," U.S. Patent No. 5,283,174. The  $T_m$  can be measured using HPA in the following manner. Probe molecules are labeled with an acridinium ester and permitted to form probe:target hybrids in a lithium succinate buffer (0.1 M lithium succinate buffer, pH 4.7, 20 mM EDTA, 15 mM aldrithiol-2, 1.2 M LiCl, 3% (v/v) ethanol absolute, 2% (w/v) lithium lauryl sulfate) using an excess amount of target. Aliquots of the solution containing the probe:target hybrids are then diluted in the lithium succinate buffered solution and incubated for five minutes at various temperatures starting below that of the anticipated  $T_m$  (typically 55°C) and increasing in 2-5°C increments. This solution is then

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diluted with a mild alkaline borate buffer (600 mM boric acid, 240 mM NaOH, 1% (v/v) TRITON® X-100 (octoxynol), pH 8.5) and incubated at an equal or lower temperature (for example 50°C) for ten minutes.